LESSON 1: IMPORTANCE AND HISTORICAL VIEW OF PLANT TISSUE CULTURE

Objective

To begin with, one should know the importance of plant tissue culture in the improvement of useful crop plants and also the ways in which it has helped mankind.

Plant tissue culture forms an integral part of any plant biotechnology activity. It offers an alternative to conventional vegetative propagation. But, tissue culture requires attention-to-detail and unless practiced as art and science, the entire process is rather unforgiving. The various objectives achievable or achieved by plant tissue culture may be summarized as under:

a. Crop Improvement

As you all understand that for any crop improvement, conventional breeding methods are employed which involve six to seven generations of selfing and crossing- over to obtain a pureline. With plant tissue culture techniques, production of haploids through distant crosses or using pollen, anther or ovary culture, followed by chromosome doubling, reduces this time to two generations.

b. Micropropagation

Plant tissue culture techniques have also helped in large- scale production of plants through micropropagation or clonal propagation of plant species. Small amounts of tissue can be used to raise hundreds or thousands of plants in a continuous process. This is being utilized by industries in India for commercial production of mainly ornamental plants like orchids and fruit trees, e.g., banana. Using this method, millions of genetically identical plants can be obtained from a single bud. This method has, therefore, become an alternative to vegetative propagation. Shoot tip propagation is exploited intensively in horticulture and the nurseries for rapid clonal propagation of many dicots, monocots and gymnosperms.

c. Genetic Transformation

Tissue culture, in combination with genetic engineering is very useful in gene transfers. For example, the transfer of a useful bacterial gene say, cry (crystal protein) gene from Bacillus thuringiensis, into a plant cell and, ultimately, regenaration of whole plants containing and expressing this gene (transgenic plants) can be achieved.

d. Production of Pathogen-free Plants

Eradication of virus has been an outstanding contribution of tissue culture technology. It was found that even in infected plants the cells of shoot tips are either free of virus or carry a negligible amount of the pathogn. Such shoot tips are cultured in a suitable culture medium to obtain virus- free plants. This technique is economical and used very frequently in horticulture, production of virus- free ornamentals etc.

e. Production of Secondary Metabolites

Cultured plant cells are also known to produce biochemicals [secondary metabolites] like, alkaloids, terpenoids, phenyl propanoids etc. of interest. The technology is now available to the industry. The commercial production of 'shikonin'[a naphthoquinone] from cell cultures of Lithospermum erythrorhizon, has been particularly encouraging.

What does the Term Tissue Culture Mean?

The term'Tissue Culture' is commonly used in a very wide sense to include in vitro aseptic culture of plant cells, tissues and organs. The in vitro cultivation of plant cells produces an unorganized mass of cells called 'Callus' which, depending upon the medium of culture gives rise to roots, somatic embryos, shoots, etc.

Another form of cell culture is, in vitro culture of single or relatively small groups of plant cells in a liquid medium. Such cultures are known as 'Suspension' Cultures'.

Sometimes, organized structures like root tips, shoot tips, embryos etc. are cultured in vitro to obtain their development as organized structures. These are called 'Organ Cultures'.

Introductory History (Refer Table 1)

Development of the science of tissue culture is historically linked to the cell and subsequent propounding of the 'cell theory'. The in vitro techniques were developed initially to demonstrate the totipotency of plant cells predicted by Haberlandt in 1902. Totipotency is the ability of a plant cell to develop into a complete plant. In 1902, Haberlandt reported culture of isolated single palisade cells from leaves in Knop's salt solution enriched with sucrose. Cells were able to synthesise starch as well as increase in size and survived for several weeks, but failed to divide. He realized that 'asepsis' (sterile) was necessary to make the cultures free from microcontamination. Haberlandt is thus regarded as the father of tissue culture.

Efforts continued to develop techniques for cultivation of plant cells under defined conditions. Brilliant contributions came from R.J. Gautheret in France and P.R. White in U.S.A. in 1985. Most of the modern tissue culture media have been derived from the work of Skoog and coworkers during 1950-60.

Embryo Culture

Hannig [1904] initiated a new line of investigation involving the culture of embryogenic tissue. He excised nearly mature embryos of some Crucifers and successfully grew them to maturity on mineral salts and sugar solution.

Winkler [1908] cultivated segments of string bean and observed some cell divisions, but no proliferation.

Embryo culture was also utilized by Laibach in 1925 to recover hybrid progeny from an interspecific cross in Linum.

Van Overbeek et al. [1941] used coconut milk (embryo sac fluid) for embryo development and callus formation in Datura which proved a turning point in the field of embryo culture.

Root Tip Culture

A new approach to tissue culture was conceived simultaneously by Kotte (Germany) and Robbins (USA) in 1922. They postulated that a true in vitro culture could be made easier by using meristematic cells, such as those that operate in the root tip or bud.

An important breakthrough for continuously growing root tip cultures came from White (1934,1937), who initially used yeast extract in a medium containing inorganic salts and sucrose but later replaced it by three B vitamins, namely, pyridoxine, thiamine and nicotinic acid. White's synthetic medium later proved to be one of the basic media for a variety of cell and tissue cultures.

Haploid Plants

Maheshwari and Guha (1964) of Botany Department, Delhi University were the first to produce Haploid plants from anther culture of Datura. This marked the beginning of anther culture or pollen culture for the production of haploid plants. The technique has been further developed by Nitsch & Nitsch who isolated microspores of tobacco to produce complete plants.

Protoplasts Culture

What are Protoplasts?

Protoplasts are naked cells from which cell wall has been removed. In1960, Cocking produced large quantities of protoplasts by using cell wall degrading enzymes. It is now possible to regenerate whole plants from protoplasts and also to fuse protoplasts of different species.

In 1972, Carlson et al., produced the first somatic hybrid plant by fusing the protoplasts of Nicotiana glauca and N. langsdorfii. Since then, many somatic hybrids have been produced.

Let us now discuss the history of various hormones that are used in the tissue culture media.

Role of Auxin

During mid-thirties, it was discovered that a successful establishment of callus cultures depended on IAA (indole-3-acetic acid), the endogenous auxin and the role of B vitamins in plant growth and in root cultures.

Gautheret, White and Nobecourt in 1939, independently established the first growing callus cultures from cambium tissue.

Gautheret (1934) cultured 'cambium cells' on the surface of a medium (Knop's solution containing glucose and cysteine hydrochloride) solidified with agar. After two months, he observed proliferation of callus from these cells. He found that addition of 'auxin' (IAA- indoleacetic acid) enhanced the proliferation of cambial cultures.

White (1939) reported similar results in the cultures from 'tumour' tissues of the hybrid Nicotiana glauca X N. langsdorfii.

Nobecourt also established continuously growing cultures of carrot slices.

Finally, Gautheret, White and Nobecourt gave the possibility of cultivating plant tissue for an unlimited period, using media enriched with auxins.

Role of Cytokinin

Steward (1948) reported for carrot explants that 'coconut milk' enhanced more proliferation of callus than did auxin. This indicated that the milk contained a stimulating substance that was not auxin.

Skoog & Tsui (1951) demonstrated that 'adenine' stimulates cell division and induces bud formation in tobacco tissue even in the presence of IAA (which normally acts as a bud inhibitor).

Skoog & Miller (1955) finally isolated a derivative of adenine (6-furfuryl aminopurine) and named it as 'Kinetin'. A substance with kinetin like properties was also found in young maize endosperm which is called as Zeatin. It was also verified that a similar substance called Ribosylzeatin occurred in coconut milk.

Now, many synthetic as well as natural compounds with kinetin- like activity are known which show bud- promoting properties. These substances are collectively called 'CYTOKINS'. These are used to induce divisions in cells of highly mature and differentiated tissues (such as mesophyll or endosperm from dried seeds), even in the presence of auxin in cultures.

Hormonal Control of Organ Formation

Before beginning with the study of plant tissue culture , it is necessary to understand the mechanism of various hormones controlling the development of different organs in vitro.

Skoog & Miller (1957) proposed the concept of hormonal control of organ formation. They used 'tobacco pith cultures' and showed that root and bud initiation depend upon a balance between auxin and kinetin. High cncentration of auxin promoted 'rooting', whereas proportionally more kinetin initiated bud or shoot formation. Unequal proportion of auxin and cytokinin led to unorganized growth of the tissue.

The determination of organogenesis also depends upon the source of plant tissue, environmental factors, composition of media, polarity, growth substances and other factors also, apart from hormonal balance only.

A General Idea about the Basic Terminology

Tissue culture is a good means for understanding the factors responsible for cell differentiation and organ formation. In plant tissue culture experiments, we use either 'single cell cultures' or 'explants'.

What is an 'Explant'?

Explants are pieces of differentiated tissues which initiate growth in cultures.

'Single cells' can be isolated either from cultured tissues or from intact plant organs.

The explant can be cultured on a medium to produce 'callus'.

What is 'Callus'?

Callus is an undifferentiated growth in solid form. It is an unorganized mass of cells.

The callus may be separated from explant and transferred to a fresh medium to get more tissue. Pieces of undifferentiated calli are transferred to liquid medium, which is continuously agitated to obtain a 'Suspension Culture'. Agitation (shaking) of pieces breaks them into smaller clumps and single cells and also

maintains uniform distribution of cells and cell clumps in the medium. It also allows gaseous exchange.

Suspension cultures with single cells can also be obtained from intact plant organs either

i. mechanically (grinding the tissue followed by cleaning, filteration and centrifugation)

ii. enzymatically (treating excised and peeled leaves with macerozyme)- as in protoplast isolation.

This is how single cells are isolated and maintained in liquid cultures.

When an explant from differentiated tissue is directly used for culture on a nutrient medium, the non- dividing, dormant cells first undergo certain changes to achieve a meristematic state (dividing state). The phenomenon of the reversion of mature cells to the meristematic state leading to the formation of callus is called as 'Dedifferentiation'.

Cells of the callus have the ability to form a whole plant, and this phenomenon is described as 'Redifferentiation'. These two phenomenon of dedifferentiation and redifferentiation take place due to 'cellular totipotency', found only in plant cells and not in animal cells. Therefore, generally a callus phase is involved before the cells can undergo redifferentiation leading to regeneration of a whole plant.

For more Definitions, follow the list given below:

Definitions for Plant Tissue Culture - Complete List

- Adventitious - in reference to a bud, embryo, root or shoot that arises in tissues and locations that are not the normal origin in the plant.
- Aseptic - free from bacteria, fungi or other microorganisms.
- Autoclave - equipment that provides heat under high steam pressure for purposes of sterilization.
- Axillary bud or shoot - a bud or shoot that arises from the axil of leaves or normal origin.
- Callus - tissue that develops as a response to injury caused by physical or chemical means; cells are differentiated but unorganized.
- Cell - a structural and physiological unit of a living organism (plant).
- DNA (Deoxyribonucleic acid) - composed of organic chemicals and is the genetic material that ultimately determines an organism's characteristics.
- Embryo - a rudimentary plant
- Epigenetic changes - persistent changes in phenotype that involve the expression of particular genes.
- Explant - the plant part that is put into tissue culture.
- Gene - specific sequence of DNA that codes for a specific trait.
- Genotype - the sum total of all genes present in an organism (plant).
- Germ - cells or tissues that are involved in reproduction and have one-half of the genetic material of somatic cells or tissues.
- Habituation - tissue cultures losing their requirements for a supply of exogenous growth regulators.
- In vitro - isolated from the living organism and artificially maintained.
- Micropropagation - the production of plants from plant parts used in tissue culture.
- Mutant - an organism (plant) that has a mutation.
- Mutation - change in the DNA sequence that is different from the original sequence.
- Organ - a distinct and visibly differentiated part of an organism.
- Organogenesis - refers to forming organs, in particular both roots and shoots from callus cells.
- Phenotype - actual appearance and behavior of an organism (plant).
- Plantlets - small complete plants that were produced via tissue culture.
- Propagules - tissue that is divided and used for further multiplication.
- Somaclonal Variation - variation (usually in phenotype and/or perhaps genotype) induced in cells by the tissue culture process.
- Somatic - cells or tissues that are vegetative and have the complete genetic material of the organism (plant).
- Subculture - divide a propagule and transfer individual parts into other culture vessels.
- Tissue - a group of cells organized into a structural and functional unit.
- Tissue culture - is the science of growing plant cells, tissues, or organs under artificial conditions.

Types of Plant Tissue Culture

Plant tissue culture, which covers all types of aseptic plant culture should be used in a restricted sense and it is possible to distinguish it into various types of cultures.

- Seed Culture - Culture of seeds in vitro to generate seedlings/plants
- Embryo Culture - Culture of isolated mature or immature embryos.
- Organ Culture - Culture of isolated plant organs. Different types can be distinguished, e.g. meristem, shoot tip, root culture, anther tissue culture
- Callus Culture - Culture of a differentiated tissue from explant allowed to dedifferentiate in vitro and a so-called callus tissue is produced.
- Cell culture - Culture of isolated cells or very small cell aggregates remaining dispersed in liquid medium
- Protoplast culture - Culture of plant protoplasts, i,e., cells devoid of their cell walls.
- Anther culture - Culture of anthers.

For a complete Historical Account, refer to Table 1:

Table.1. A Historical Account of Milestones in the Field of Plant Tissue and Cell Culture (Adapted from: White, 1963; Pierik, 1987; Brar and Khush, 1994; Endress, 1994).

Year	Important Discoveries and their References
1838 & 1839	Cell theory, suggesting totipotentiality of cells. Schleiden M. J., Arch. Anat., Physiol. U. wiss. Med. (J. Muller), 1838: 137-176; Schwann T., W. Engelman, No. 176 (1910).
1882	Plants synthesize organ-forming substances that are polarly distributed. Sachs J., Arch. Bot. Inst. Wurzburg, 2: 453 & 689. A
1902	First but unsuccessful attempt of tissue culture using monocots. Haberlandt G., Sitzungsber Akad. Wiss. Wien, Math.-Naturwiss. Kl., 111: 69-92.
1904	First attempt in embryo culture of selected Crucifers. Hannig B., Bot. Zeitung, 62: 45-80.
1909	Fusion of plant protoplasts though the products failed to survive. Kuster E., Ber. Dtsch. Bot. Ges., 27: 589-598.
1921	Cultivation of fragments of plant embryos. Molliard M., C. R. Soc. Biol. (Paris), 84: 770-772.
1922	Asymbiotic germination of orchid seeds. Knudson L., Bot. Gaz., 73: 1-25.
	In vitro culture of root tips. Robbins W. J., Bot. Gaz., 73: 376-390.
1924	Callus formation on carrot root explants by use of lactic acid. Blumenthal F. and Meyer P. Z. Krebsforsch. 21: 250-252.
1925	Embryo culture for interspecific crosses in Linum spp. Laibach F., Z. Bot., 17: 417-459.
	Symbiotic germination of orchid seeds. Knudson L., Bot. Gaz., 29: 345-379.
1929	Embryo culture to avoid cross incompatibility in Linum spp. Laibach F., J Hered., 20: 201-208.
1934	In vitro culture of cambial tissues of different trees and shrubs failed. Guatheret R. J., C. R. Acad. Sci. (Paris), 198: 2195-2196.
	Successful long-term culture of tomato roots. White P. R., Plant Physiol., 9: 585-600.
	Identification of the first plant hormone, IAA, leading to cell enlargement. Kogl F. et al., Z. Physiol. Chem., 228: 90-103.
1936	Embryo culture of different gymnosperms. LaRue C. R., Bull. Torrey Bot. Club, 63: 365-382
1939	Successful continuously growing cambial cultures of carrot and tobacco. Gautheret R. J., C. R. Acad. Sci. (Paris), 208: 118-120; Nobecourt P., C. R. Soc. Biol. (Paris), 130: 1270-1271; White P. R., Am. J. Bot., 26: 59-64.
1940	Culture of cambial tissue of Ulmus to study adventitious shoot formation. Gautheret R. J., C. R. Acad. Sci., 210: 632-634.
1941	Coconut Milk used for growth and development of very young Datura embryos. Overbeek J. van et al., Science, 94: 350-351.
1942	Observation of secondary metabolites in plant callus cultures. Gautheret R. J. Bull. Soc. Chim. Biol. 41: 13.
1943-1950	Tumor-inducing principle of crown gall tumors identified. Braun A. C. Phytopathol. 33: 85-100 & P. N. A. S. USA 45: 932-938.
1944	First In vitro culture of tobacco used to study adventitious shoot formation. Skoog F., Am. J. Bot., 31: 19-24.
1945	Cultivation of excised stem tips of Asparagus. Loo S. W., Am. J. Bot., 32: 13-17.
1946	First whole plants of Lupinus and Tropaeolum from shoot tips. Ball E., Am. J. Bot., 33: 301-318.
1948	Formation of adventitious shoots and roots in tobacco. Skoog F. and Tsui C., Am. J. Bot., 355: 782-787.
1949	Culture of fruits In vitro. Nitsch J. P., Science, 110: 499.
1950	Organs regenerated from callus of Sequoia. Ball E., Growth, 14: 295-325.
	First successful cultures of Monocots using coconut milk. Morel G. C. R. Acad. Sci., 230: 2318-2320.
1951	Culture of excised ovaries In vitro. Nitsch J. P., Am. J. Bot., 38: 566-577.
	Chemical control of growth and organ formation in culture demonstrated. Skoog F., Annee Biol., 26: 545-562.
1952	Virus-free Dahlia through meristem culture. Morel G. and Martin C., C. R. Hebd. Seances Acad. Sci. (Paris), 235: 1324-1325.
	First successful micro-grafts. Morel G. and Martin C., C. R. Acad. Sci. (Paris), 235: 1324-1325.
1953	Haploid callus from pollen grain of Ginkgo biloba. Tulecke W. R.., Science, 117: 599-600.
1954	First calli produced from a single cell by use of nurse cultures. Muir W. H. et al., Science, 119: 877-878.
1955	Discovery, structure and synthesis of Kinetin. Miller C. et al., J. Am. Chem. Soc., 77: 1392 & 2662-2663.
1956	In vitro cultivation of normal and tumor tissues of Picea glauca. Reinert J. and White P. R., Physiol. Plant., 9: 177-189.

1956	US patent NO. 2747334 for: Production of substances from plant tissue culture of Phaseolus by Routien J. B. and Nickell L. G.
1957	Discovery that root or shoot formation in culture depends on auxin : cytokinin ratio. Skoog F. and Miller C. O., In vitro Symp. Soc. Exp. Biol., No. 11: 118-131.
	Culture of excised anthers of Allium cepa. Vasil I. K., Phytomorph., 7: 138-149.
1958	In vitro culture of excised ovules of Papaver somniferum. Maheshwari N., Science, 127: 342.
	Regeneration of somatic embryos from nucellus of Citrus ovules. Maheshwari P. and Rangaswamy N. S., Ind. J. Hort., 15: 275-281.
	Pro-embryo formation in callus clumps and cell suspension of carrot. Reinert J. and Steward F. C., Naturwiss., 45: 344-345.
	Growth and development in suspension cultures. Steward F. C. et al., Am. J. Bot., 45: 693-708.
1959	Production of large amounts (134 L) of plant tissue by submerged culture. Tulecke W. and Nickell L. G., Science, 130: 863-864.
1960	First test tube fertilization in Papaver rhoeas. Kanta K., Nature, 188: 683-684.
	Enzymatic degradation of cell wall for protoplast formation. Cocking E. C., Nature, 187: 927-929.
	Vegetative propagation of orchids by meristem culture. Morel G., Am. Orchid Soc. Bull., 29: 495-497.
1962	Development of MS medium. Murashige T. and Skoog F., Physiol. Plant., 15: 473-497.
	In vitro flower induction in tobacco Aghion D., C. R. Acad. Sci., 255: 993-995.
1964	First haploid plants from Datura androgenesis. Guha S. and Maheshwari S. C., Nature, 204: 497 and Nature, 212: 97-98 (1966).
	Regeneration of roots and shoots on callus of Populus tremuloides. Mathes M. C., Phyton, 21: 137-141.
1965	Differentiation of tobacco plants from a single isolated cell in microculture. Vasil V. and Hildebrandt A. C., Science, 146: 76-77 & 150: 889-892.
	Protocorm formation in orchids In vitro. Morel G., Cymbidium Soc. News, 20: 3
1967	Flower induction in Lunaria annua by vernalization In vitro. Pierik R. L. M., See Pierik R. L. M., (1987) In vitro Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht.
1967	Yields of secondary products in cell culture equal to those of intact plants of Ammi visnaga. Kaul B. and Staba E. J., Planta Med., 15: 145-156.
	Haploid plants from pollen grains of tobacco. Bourgin J. P. and Nitsch J. P., Ann. Physiol. Veg., 9: 377-382 & 10: 69-81.
1969	Protoplast isolation from suspension culture of Hapopappus gracilis. Ericksson T. and Jonassen K., Planta, 89: 85-89
1970	Selection of biochemical mutants in tobacco. Carlson P. S., Science, 168: 487-489.
	Hybrid embryo culture and subsequent chromosome elimination for haploid production in Barley. Kasha K. J. and Kao K. N., Nature, 225: 874-875
	Protoplast fusion. Power J. B. et al., Nature, 225: 1016-1018.
1971	Plant regeneration from mesophyll protoplasts of tobacco. Takebe I. Et al., Naturewiss., 58: 318-320.
1972	Interspecific hybridization of Nicotiana spp. using protoplasts. Carlson P. S. et al., P. N. A. S. (USA), 69: 2292-2294.
1973	Cytokinins found to be capable of breaking dormancy in Gerberas. Pierik R. L. M. et al., Sci. Hort., 1: 117-119.
1974	Induction of branching by cytokinins in Gerbera shoot tips Murashige F. et al., Hortsci., 9: 175-180.
	Regeneration of haploid Petunia plants from protoplasts Binding R. J., Z. Pflanzenphysiol., 101: 119-130.
	Fusion of haploid protoplasts to form polyploids. Melchers G. and Lalib G., Mol. Gen. Genet. 135: 277-294.
	Bio-transformation in PTC Reinhard E., In: Tissue culture and Plant science, Street H. E. (ed.), Acad Press NY,: 433-459.
	Ti plasmid as the tumor inducing principle in crown gall. Zaenen I. Et al., J. Molec. Biol., 86: 109-127; Larebeke N. van et al., Nature, 252: 169-170.
1975	Positive selection of maize callus culture resistant to Helminthosporium maydis. Gengenbach B. G. and Green C. E., Crop Sci., 15: 645-649
1976	Shoot induction from cryo-preserved shoot tips of carnation. Seibert M., Science, 191: 1178-1179.
	Protoplast fusion of Petunia hybrida with P. parodii. Power J. B. et al., Nature, 263: 500-502.
	Octopine and Nopaline synthesis and breakdown is regulated by Ti plasmid. Bomhoff G. et al., Molec. Gen. Genet., 145: 177-178.

1977	Successful integration of T-DNA in plants. Chilton M. D. et al., Cell, 11: 263-271.
	Cultivation of tobacco cells in 20,000 L bioreactors. Noguchi M. et al., Plant Tissue Culture & its Biotechnological Application, Springer Verlag, Berlin,: 85-94.
	Development of two-stage culture medium for suspension cell cultures. Zenk M. H. et al., Plant Tissue Culture & its Biotechnological Application. Springer Verlag, Berlin,: 27-43.
1978	Somatic hybridization of tomato and potato. Melchers G. et al., Carlsburg Res. Comm., 43: 203-218.
	Industrial scale fermentation of plant cells for production of shikonin. (Selection of cell lines with higher yield of secondary products). Tabata M. et al., Frontiers of Plant Tissue Culture 1978, Univ. Calgary Press, Calgary,: 213-222.
1979	Alginate beads used for plant cell immobilization for biotransformation and secondary metabolite production. Brodelius P. et al., FEBS Lett., 103: 93-97.
	Co-cultivation procedure developed for the Agrobacterium mediated transformation of protoplasts. Marton L. et al., Nature, 277: 129-131
1980	The use of immobilized cells for bio-transformation of digitoxin intro digoxin. Alfermann A. W. et al., Planta Medica, 40: 218.
1981	Introduction of the term somaclonal variaion. Larkin P. J. and Scowcroft W. R., Theor. Appl. Gen., 60: 197-214.
	Isolation of auxotrophs by cell colony screening in haploid protolasts of Nicotiana plumbaginifolia treated with mutagens. Sidorov V. et al., Nature, 294: 87-88.
	Use of a hollow fiber reactor for secondary metabolite production. Shuler M. L., Ann. NY Acad. Sci., 369: 65-79.
1982	Naked DNA transformation of protoplasts. Krens F. A. et al., Nature, 296: 72-74.
	Electrofusion of protoplasts Zimmermann U., Biochim. Biophys. Acta, 694: 227-277.
1983	Intergeneric cybrid in radish and rape. Pelletier G. et al., Molec. Gen. Genet., 191:244-250.
	First industrial production of secondary metabolites by suspension cultures of Lithospermum spp. by Mitsui Petrochemicals.
	Beneficial use of elicitors in cell suspension cultures. Wolters B. and Eilert U. Dtsch. Apoth. Zeitg., 123: 659-667.

1983	Co-integrate type of vectors designed for Agrobacterium transformation. Zambryski P. et al., EMBO J., 2: 2143-2150.
1984	Transformation of Nicotiana protoplasts with plasmid DNA and regeneration of transformed plants. Paszkowski J. et al., EMBO J., 3: 2717-2722.
1985	Infection and transformation of leaf discs with Agrobacterium tumefaciens and regeneration of transformed plants. Horsch R. B. et al., Science, 227: 1229-1231.
	Development of disarmed Ti plasmid vector system for plant transformation. Fraley R. T. et al., Bio/Technol., 3: 629-635.
	Development of binary vector system for plant transformation. An G. et al., EMBO J., 4: 277-284.
	Gene transfer in protoplasts of Dicot and Monocot plants by electroporation. Fromm M. E., P. N. A. S. (USA), 82: 5824-5828.
	Hairy root production for the first time in Hyoscyamus muticus. These roots produced more hyoscyamine than in planta. Flores H. E. and Filner P., Primary & Secondary Metabolism of Plant Cell Cultures. Springer Verlag, (Eds. Neumann K. H., Barz W. and Reinhard E.): 174-186.
1986	Transformation of tobacco protoplasts by direct DNA microinjection. Crossway A. et al., Mol. Gen. Genet., 202: 179-185.
1987	Use of Microprojectile gun for particle bombardment for genetic transformation and recovery of individuals showing transient gene expression. Klein T. M. et al., Nature, 327: 70-73.
	First monocot (Asparagus) transformation by Agrobacterium tumefaciens. Bytebier B. et al., P. N. A. S. (USA), 84: 5345-5349.
	Micro-injection for direct DNA delivery into plant cells. Miki B. L. A. et al., Plant DNA Infectious Agents., vol. 3: 249-265.
1988	Recovery of stable transformants through particle bombardment. Klein T. M. et al., P. N. A. S. (USA), 85: 4305-4309.
	Automated mass propagation with organogenesis and embryogenesis. Levi R. et al., Biotechnol., 6: 1035.
1990	Plant transformation by microinjection of intact plant cells. Neuhaus G., Physiol. Plant., 79: 213-217.
	Electroporation of intact plant tissues for direct DNA delivery. Dekeyser R. A. et al., Plant Cell, 2: 591-602.

	Silicon carbide fiber-mediated DNA delivery in plant cells. Kaeppler H. F. et al., Plant Cell Rep., 9: 415-418.
1991	Cryopreservation of alkaloid-producing cell culture of Catahranthus. The cells retain the property of alkaloid synthesis even after thawing. Lynch P. T. and Benson E. E., Rice Genetics II, IRRI, Manila, Phillipines,: 321.
	Production of first transgenic plants of a conifer (Larix decidua, by Agrobacterium rhizogenes mediated transformation). Huang Y. et al., In vitro Cell Dev. Biol., 27: 201-207.
1992	Successful metabolic engineering of Atropa belladona for increased alkaloid production. Yun D.-J. et al., P. N. A. S. (USA), 89: 11799-11803.
	Herbicide resistant rice plants through PEG mediated transformation of protoplasts. Dutta S. K. et al., Plant Mol. Biol., 20: 619-629.
1993	In vitro fertilization with isolated single gametes resulting in zygotic embryogenesis and recovery of fertile maize plants. Kranz E. and Lorz H., The Plant Cell, 5: 739-746.
	"Green Hairy roots" showing photoautotrophy due to development of photosynthetic ability. Flores H. E. et al., Plant Physiol., 101: 363-371.
1994	Marketing of genetically engineered tomato: 'Flavr-Savr'
	Improved plant transformation through electrophoresis. Giesbach R. J., Plant Sci., 102: 81-89.
1996	Development of 'agrolistic' method of plant transformation. Hansen G. and Chilton M. D., P. N. A. S. (USA), 93: 14978-14983. Development of a binary bacterial artificial chromosome (BBIC) vector for Agrobacterium-mediated transformation (Transfer capacity of 150 kb). Hamilton C. M. et al., P. N. A. S. (USA), 93: 9975-9979.

Conclusion

This Lesson gives a complete historical background on the pioneering research work done in plant tissue culture. The updated history has been supplemented with definitions and terms used in tissue culture so that the terms are familiar to you before initiating studies on the subject. Importance of tissue culture has also been given to some extent, although details on these will be undertaken in the forthcoming Lessons.

Questions

1. What are the various objectives achieved by plant tissue culture?
2. Give a historical background of the role of growth regulators (hormones) in plant tissue culture.
3. Give contributions of the following:
   a. Haberlandt
   b. Hanning
   c. Laibach
   d. Gautheret
   e. Steward
   f. Skoog and Miller

Note